Original

The Combined Effects of Sodium Fluoride and Pyridoxine Hydrochloride :

II. *in vivo* Studies in Animals*

フッ化ナトリウムと塩酸ピリドキシン併用の有用性

II. 動物を用いた *in vivo* における検討

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概要：我々は、従来のフッ化物よりもさらに優れたう蝕予防効果を有する物質を検索する目的で、フッ化ナトリウム (NaF) との併用によりその効果を増強させる作用を有する物質について検討した。その結果、酸性条件下 (pH 4.5) で NaF と塩酸ピリドキシン (VB₆・HCl) とを併用すると、合成ハイドロキシアパタイト粉末 (synthetic HA) へのフッ素取り込み、耐酸性、結晶性が向上し、牛歯エナメル質へのフッ素取り込みも向上することを明らかにした¹⁾。そこで次に、NaF と VB₆・HCl の併用によるう蝕抑制効果をラット実験う蝕系において検討した。う蝕原性 *Streptococcus sobrinus* 6715株を定着させたラットをう蝕誘発性飼料 Diet #2000で63日間飼育した。実験期間中を通じて NaF+VB₆・HCl 水溶液、NaF 水溶液、生理食塩液を1日1回、週5回ラットの上顎臼歯部に塗布し、ブラークスコア、カリエススコア、う蝕裂溝数、臼歯へのフッ素の取り込みについて比較検討した。

結果の概要は次の通りである。

- 1) NaF+VB₆・HCl 溶液塗布群および NaF 溶液塗布群はコントロール群 (生理食塩液塗布) に比べブラークスコアは有意に低かった。
- 2) NaF+VB₆・HCl 溶液塗布群および NaF 溶液塗布群はコントロール群に比べカリエススコアは有意に低かった。
- 3) NaF+VB₆・HCl 溶液塗布群および NaF 溶液塗布群はコントロール群に比べう蝕裂溝数は有意に少なかった。さらに、NaF+VB₆・HCl 溶液塗布群は NaF 溶液塗布群に比べう蝕裂溝数は有意に少なかった。
- 4) NaF+VB₆・HCl 溶液塗布群および NaF 溶液塗布群の臼歯歯冠部はコントロール群に比べ有意に高

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特別掲載

いフッ素濃度を示した。さらに、 $\text{NaF} + \text{VB}_6 \cdot \text{HCl}$ 溶液塗布群の臼歯歯冠部は NaF 溶液塗布群に比べ有意に高いフッ素濃度を示した。

以上の *in vivo* における検討結果より、 NaF と $\text{VB}_6 \cdot \text{HCl}$ の組み合わせはう蝕予防に対して非常に有用であると考えられた。

Key words: Experimental Caries, Plaque Score, Caries Score, Fissure Caries, Fluoride Uptake

索引用語: 実験う蝕, プラークスコア, カリエスコア, 裂溝う蝕, フッ素取り込み

Introduction

At present, fluoride has widely contributed to caries prevention by the methods of water fluoridation, fluoride painting, fluoride mouth rinsing, etc²⁻⁴⁾. Among these methods, fluoride dentifrice is one of the most popular. The fluoride compounds added to dentifrice are mainly sodium fluoride (NaF), sodium monofluorophosphate, or stannous fluoride, and their caries preventing effects have been proven²⁻⁴⁾. On the other hand, in Europe, organic amine fluoride compounds were developed and have been used in dentifrices. Their excellent effects have been recognized by *in vitro*, *in vivo* and clinical experiments⁵⁻⁸⁾.

In a previous study, we searched for more effective caries preventive substances combined with NaF using synthetic hydroxyapatite and bovine enamel. A remarkable enhancing effect of NaF in combination with $\text{VB}_6 \cdot \text{HCl}$ at pH 4.5 was recognized¹⁾. The purpose of this study was to elucidate the *in vivo* effect of NaF in combination with $\text{VB}_6 \cdot \text{HCl}$, using experimental caries in rats, in comparison with NaF only.

Materials and Methods

The experimental design is shown in Fig. 1.

—Experimental animals and feeding—

Sixty 12-day-old Wistar rats, thirty two males and twenty eight females, were obtained from Shizuoka Agr. Corp. for Exp. Animals (Shizuoka, Japan). At 15 days of age, all animals were compulsorily weaned by feeding with standard power diet, and after weaning (18 days), they were fed cariogenic Diet# 2000 (CLEA Japan) until the end of the experiment (81 days). The diet and distilled water were provided *ad libitum* throughout the experiment. Before weaning, each litter of rats was kept in a plastic cage with its mother, and after weaning, they were separated in stainless cages. The environment was maintained constant at a temperature of $23 \pm 3^\circ\text{C}$, a relative humidity of $50 \pm 10\%$, and 12:12 hour light-dark cycle. The rats were weighed once a week to check their health.

—Bacteriological methods—

Streptococcus sobrinus strain 6715 (serotype g, resistant to streptomycin) was kindly provided by Dr. T. Koga, NIH, Tokyo. Test strains were grown in 21 of BHI broth (Eiken Chemical Co., Ltd) anaerobically at 37°C overnight, centrifuged (5000 g, 30 min, 4°C) and suspended in 20 ml sterile saline. This liquid included about 1.0×10^{10} cells per ml. The indigenous oral flora was suppressed by providing drinking water containing penicillin G potassium (4000 U/ml, Banyu Pharmaceutical Co., Ltd) and a diet containing tetracycline hydrochloride (4 mg/g, Wako Pure Chemical Industries, Ltd) for three days (15-17 day-old). Bacterial inoculations were started at 18 days of age. 0.1 ml of the bacterial suspension was applied to the rat mouth with a syringe for six days. Colonization tests were performed on MS-agar just before inoculation and after every two weeks.

—Groups—

At 24 days of age, the rats were divided into three groups: ① $\text{NaF} + \text{VB}_6 \cdot \text{HCl}$ (900 ppm of F, 0.97 %

Fig. 1 Experimental design

VB₆·HCl, pH 4.5) group, ②NaF (900 ppm of F, pH 4.5) group, ③control (saline) group.

—Application of fluoride—

At 25 days of age, rats in the three groups were given 3 minutes of topical application of each fluoride solution or saline once a day, five days a week for eight weeks. The test solutions were applied to the maxillary and mandibular molar teeth with a small cotton swab under anaesthesia⁹.

—Scoring of dental caries—

Plaque accumulation on the surface of molar teeth was evaluated by the method of Saito *et al.*¹⁰ (Table 1). Caries extent in molar teeth was evaluated according to the method of Takei *et al.*¹¹, with some modifications. 22 Specific occlusal fissures were examined under the optical microscope with the aid of a explor and scored according to Table 2, and total scores were regarded as caries score. The number of caries fissures (>grade 1) were compared among the 3 groups.

—Determination of fluoride—

After the caries scoring, all of the molar teeth were extracted and their roots were cut off. The crowns were pooled and ground into power. Fluoride in the crowns was collected by the microdiffusion technique¹² and measured with a fluoride ion electrode (ORION 96-09).

Results

—Growth of animals—

The body weight in each group during the experimental peroid, as shown in Fig. 2, increased satisfactorily, and no significant differences were recogndzed among the 3 groups. No symptom of any disease and no change in diet intake was recongized throughout the experimental period.

—Plaque accumulation—

The plaque score in each group is shown in Table 3 and Fig. 3. In the two fluoride groups, plaque accumulation was significantly ($p < 0.01$) prevented compared with the control group. However, no significant difference was seen between the NaF and NaF+VB₆·HCl group.

Table 1 Criteria of plaque accumulation

Grade

- 0 : No detectable plaque
- 1 : Plaque covering not more than 1/3 of the tooth surface
- 2 : Plaque covering 1/3~1/2 of the tooth surface
- 3 : Plaque covering more than 2/3 of the tooth surface

Table 2 Criteria of caries detection and classification of caries grades

Grade

- 0 : No detectable caries
- 1 : Enamel caries, such as white spot or stained lesion detectable with explorers
- 2 : Incipient dentin caries, such as obvious soft dentin the fissure
- 3 : Developed dentin caries, such as the lesion spread over more than 2 fissures, and to the pulp
- 4 : More developed caries than grade 3
- 5 : Remarkable destruction of the dental crown

Table 3 Effects of NaF+VB₆·HCl on plaque accumulation and caries in rats (Mean±SD)

Group	Plaque score	Caries score	Caries fissures	Fluoride uptake
Control (n=20)	20.42±4.2	39.3±14.8	18.2 ± 3.0	22.1± 3.1
NaF (n=19)	16.8 ±2.5	19.5± 6.3	13.2 ± 2.9	534.0±111.0
NaF+VB ₆ ·HCl(n=18)	16.0 ±3.0	17.4± 9.6	10.4 ± 4.9	795.4±210.5

n.s.: not significant *: p<0.05 **: p<0.01 ***: p<0.001

Fig. 2 Changes of body weight in rats **Fig. 3** Plaque scores in each group **Fig. 4** Caries scores in each group

—Caries incidence and extent—

The caries score in each group is shown in Table 3 and Fig. 4. The caries scores of the two fluoride groups were significantly ($p<0.001$) lower than that of the control group. However, there was no significant difference between the NaF and NaF+VB₆·HCl group. The number of caries fissures in each group is shown in Table 3 and Fig. 5. As shown in Table 3 and Fig. 5, the caries fissures of the two fluoride groups were significantly ($p<0.001$) fewer than in the control group. Moreover, the number of caries fissures of the NaF+VB₆·HCl group was significantly ($p<0.05$) fewer than in the NaF group.

—Fluoride uptake in molar teeth—

Fluoride concentration in the molar crowns of each group is shown in Table 3 and Fig. 6. Fluoride concentration in the teeth of NaF group was twelve times or more that of the control group. Fluoride

Fig. 5 The number of caries fissures in each group

concentration in the teeth of the NaF+VB₆·HCl group was significantly ($p<0.05$) higher than in the NaF painting group.

Discussion

The cariogenic conditions of the rats in this study were ①being fed cariogenic Diet # 2000, ②compulsory weaning early in the experimental period, and ③suppression of indigenous oral flora and inoculation with *St. sobrinus*. Fluoride concentration in the experimental solution was 900 ppm, similar to the concentration usually used in dentifrices.

—Plaque accumulation—

Dental plaque formed by oral microorganisms is an important factor of caries induction. Plaque-forming streptococci, for example, *Streptococcus mutans* or *Streptococcus sobrinus*, synthesize the insoluble glucan from sucrose and form dental plaque^{13,14}. At the same time, they produce organic acids by anaerobic glycolysis of sucrose, dissolve the enamel surface, and induce the caries^{13,14}.

The prevention of the plaque accumulation by application of fluorides is mainly assumed to be due to the ability of fluorides to inhibit the activity of enzymes in microorganisms¹⁵. The fluoride concentration which inhibits the activity of microorganism was about 30 ppm *in vitro*¹⁶.

It has been reported that glucose metabolism of *Streptococcus salivarius* was suppressed by 2ppm fluoride¹⁷. In the present study, the plaque accumulation in the fluoride groups was significantly prevented compared with the control group (Fig. 3). Although *in vitro* effects are not always seen in *in vivo* cases, in the present study the enzyme activity was inhibited by 3 minutes topical application of 900 ppm fluoride for 56 days, and the formation of plaque was prevented.

—Caries incidence and extent—

In the present study, caries scores in the two fluoride groups were significantly lower than in the control group (Fig. 4). But there was no significant difference between the NaF group and the NaF+VB₆·HCl group. It has been assumed that the mechanism of caries prevention by application of fluoride is the production of fluorapatite (FA) on the enamel surface followed by an enhanced acid resistance of the enamel^{14,16}. However, once the caries extends to the dentin or pulp, fluoride does not prevent caries. Therefore, it is thought that fluoride prevents caries incidence rather than caries progress. In this study, we detected pulp caries not only in the control group but also in the NaF and NaF+VB₆·HCl groups. This fact seemed to be one reason why there was no difference in caries score between the NaF group and the NaF+VB₆·HCl group. Next, we looked at the prevention of caries incidence, and compared the number of caries fissures. Caries fissures in the NaF+VB₆·HCl group were significantly fewer than in the NaF group. These results, we concluded that the combination of NaF and VB₆·HCl was more available for prevention of caries incidence than the separate use of NaF.

—Fluoride uptake in molar teeth—

As mentioned above, the increment of fluoride concentration in teeth by topical application is based on the production of the acid resistance FA in enamel surface. In the present study, after topical application of fluoride for 56 days, fluoride uptake in molar teeth was significantly increased. Fluoride uptake in teeth of the NaF+VB₆·HCl group was higher than in the NaF group. In our previous study, which investigated the fluoride uptake in synthetic HA and bovine enamel, fluoride uptake of the NaF+VB₆·HCl

Fig. 6 Fluoride contents in rat's molars in each group

treated group was significantly higher than in the NaF treated group¹⁾. We considered that the combination of NaF and VB₆·HCl caused the higher fluoride uptake and FA formation. In this study, we believe that this effect was amplified by the repeated application of fluoride to the enamel surface.

Conclusion

The combined effects of NaF and VB₆·HCl on the prevention of caries in rats were studied. Results were as follows:

- 1) Plaque scores of the NaF and NaF+VB₆·HCl group were lower than in the control group. No significant difference was seen between the NaF and the NaF+VB₆·HCl group.
- 2) The caries scores of the NaF and NaF+VB₆·HCl groups were lower than in the control group. No significant difference was seen between the NaF and NaF+VB₆·HCl group.
- 3) Caries fissures in the NaF and NaF+VB₆·HCl groups were significantly fewer than in the control group. Caries fissures in the NaF+VB₆·HCl group were also significantly fewer than in the NaF group.
- 4) Fluoride uptake in molar teeth was significantly increased by topical application of fluoride. Fluoride uptake in the NaF+VB₆·HCl group was significantly higher than in the NaF group.

The combination of NaF and VB₆·HCl appears to be very useful for caries prevention.

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